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TECHNICAL NOTE/TECHNISCHE NOTIZ

Alcoholic versus Aqueous Calibrators for the Enzymatic Assay of Serum Cholesterol

Alkoholische oder wäßrige Standardlösungen für die enzymatische Bestimmung von Cholesterin im Serum?

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There are conflicting reports (1—4) on the calibrating solutions (aqueous or alcoholic), used in the direct enzymatic assay (4) of serum cholesterol. In our laboratory, the influence of some alcohols in the enzymatic reaction was studied.

Aqueous cholesterol calibrators (50 µl) (Preciset, Boehringer-Mannheim), water for the blanks (50 µl), cholesterol reagent (5000 µl) (CHOD-PAP high performance, Boehringer-Mannheim), and variable volumes (0—500 µl) of alcohols were mixed. Volumes were made up to 5500 µl (with the reagent) and the mixtures were read at 495 nm or scanned (400—650 nm), as required. Four alcohols (methanol, ethanol, 1-propanol and 2-propanol) were included in the study. 1-propanol and 2-propanol, caused increases in blank absorbance (proportional to the volume fraction of the alcohol), due to turbidity. Therefore, in spite of the favourable physico-chemical properties of these two alcohols (high boiling point, high relative density and high cholesterol solubility), they were excluded from further study. Since ethanol exhibited more favourable physico-chemical properties than methanol, its effects on the reaction were further investigated. No effect on blank absorbance or turbidity was observed up to volume fractions of ethanol of 0.09. However, significant increase in standard absorbance was recorded as the result of increasing alcohol concentration, and a small shift of the absorbance peak (from 497 to 493 nm) was also observed. Calibration curves (in the range 0—10.4 mmol/l) were linear both in the absence ($r^2 = 0.99993$) and in the presence ($r^2 = 0.99996$) of ethanol, slope values being shifted from 0.0597 ± 0.0001, in the absence of ethanol, to 0.0645 ± 0.0001 in the presence of ethanol, volume fraction 0.09.

From the regression equation it was calculated that, for a sample (or standard) volume fraction of 0.01 (as usually employed), a proportional bias as low as −0.6% is introduced by the presence of ethanol in the standard, but not in the sample, when readings are taken at the peak. However, because of a small modification in the shape of the absorption spectrum, the bias is practically 0 at 546 nm, a wavelenght frequently used in clinical analysis. A significantly higher bias (−1.3%) is introduced when 2-propanol is used (4); furthermore, one occasional batch of 2-propanol was found to be heavily contaminated with peroxide, which was completely removed by distillation.

It seems reasonable to conclude that ethanolic cholesterol solutions can be used as calibrators in the direct enzymatic cholesterol assay. There are two advantages: a real primary calibrator (pure substance in chemically defined matrix) is used, and the same calibrator can be used in comparison experiments (with the proposed reference method, for instance), thus avoiding the possible introduction of bias by the use of different calibrators. Different behaviour of different aqueous calibrators in the enzymatic cholesterol assay has been reported (5).

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